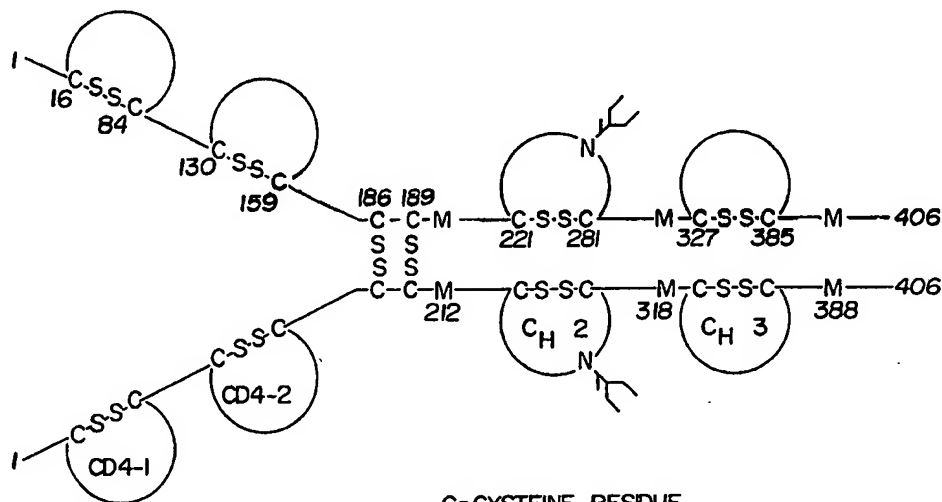




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12N 15/12, C07K 13/00 C12N 15/62, A61K 39/21, 39/42 A61K 37/02	A1	(11) International Publication Number: WO 92/21755 (43) International Publication Date: 10 December 1992 (10.12.92)
(21) International Application Number: PCT/US92/03138 (22) International Filing Date: 15 April 1992 (15.04.92) (30) Priority data: 709,006 31 May 1991 (31.05.91) US (71) Applicant: GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US). (72) Inventor: AMMANN, Arthur, J. ; 104 Dominican Drive, San Rafael, CA 94901 (US). (74) Agents: DREGER, Ginger, R. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).		(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>

(54) Title: TREATMENT OF HIV-ASSOCIATED IMMUNE THROMBOCYTOPENIC PURPURA

C = CYSTEINE RESIDUE
 -S-S- = DISULFIDE BOND
 M = METHIONINE RESIDUE
 = GLYCOSYLATION SITE
 N = ASPARAGINE RESIDUE

(57) Abstract

The invention relates to a method for treating HIV-associated immune thrombocytopenic purpura (ITP) which comprises administering to a patient in need of such treatment a therapeutically effective amount of a molecule comprising an amino acid sequence capable of binding to HIV.

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TREATMENT OF HIV-ASSOCIATED IMMUNE THROMBOCYTOPENIC PURPURA**Field of the Invention**

This application relates to the treatment of human immunodeficiency virus (HIV)-associated Immune Thrombocytopenic Purpura (ITP).

5 Background of the Invention

Thrombocytopenia is defined as a platelet count below 150×10^9 per liter, and can be due to a number of different disorders, including, for example, impaired production of platelets by the bone marrow, platelet sequestration due to splenomegaly, or increased destruction of platelets in the peripheral circulation. Based upon their underlying cause, the thrombocytopenias can be divided into different categories [Schafer, A.I., "Thrombocytopenia and Disorders of Platelet Function" pp. 1041-1049 in Internal Medicine, 3rd Edition, John J. Hutton *et al.*, Eds., Little Brown and Co., Boston/Toronto/London, 1990]. One of these categories, immune (autoimmune) thrombocytopenic purpura (ITP) is an uncommon illness caused by the production of antibodies to platelets and the subsequent destruction of platelets by the reticuloendothelial system. The autoantibody is usually IgG although other immunoglobulins have also been reported. The clinical bleeding manifestations of ITP depend on the severity of the condition, and possible associated coagulation effects. In severe cases, bleeding can be life threatening [Morris *et al.*, Ann. Int. Med. **96**, 714-717 (1982)]. Chronic ITP is usually accompanied by increased megakaryocytes in the bone marrow and frequently by an elevated platelet-associated immunoglobulin G (PAIgG) [Bussel *et al.*, Blood **62-2**, 480-486 (1983)].

ITP is also a common complication of HIV infection [Morris *et al.*, Ann. Intern. Med. **96**, 714-717 (1982)], which can occur at any stage of its natural history, both in patients diagnosed with the Acquired Immune Deficiency Syndrome (AIDS), those with AIDS-related complex, and those with HIV infection but without AIDS symptoms.

AIDS is a transmissible disease characterized by a profound deficiency of cellular immune function and the occurrence of opportunistic infection and malignancy. In 1983, a retrovirus was identified in association with this syndrome [Barre-Sinoussi, F., Chermann, J.C., Rey, F. *et al.*, Science **220**, 868-871 (1983); and Gallo, R.C., Salahuddin, S.Z., Popovic, M., *et al.*, Science **224**, 500-503 (1984)]. Subsequently, there have been numerous isolations of related retroviruses from patients in different geographical areas, and the etiologic role of this retrovirus has been clearly established. Whereas initially these isolates were variously referred to as lymphadenopathy associated virus (LAV), human T cell lymphotropic virus-III (HTLV-III) or AIDS associated retrovirus (ARV), today the accepted terminology is human immunodeficiency virus (HIV), which has more subtypes, e.g. HIV type-1 (HIV-I) and HIV type-2 (HIV-2).

The primary immunologic abnormality resulting from infection by HIV is the progressive depletion and functional impairment of T lymphocytes expressing the CD4 cell surface

glycoprotein (H. Lane *et al.*, Ann. Rev. Immunol. **3**:477 [1985]). CD4 is a non-polymorphic glycoprotein with homology to the immunoglobulin gene superfamily (P. Maddon *et al.*, Cell **42**:93 [1985]). Together with the CD8 surface antigen, CD4 defines two distinct subsets of mature peripheral T cells (E. Reinherz *et al.*, Cell **19**:821 [1980]), which are distinguished by their ability to interact with nominal antigen targets in the context of class I and class II major histocompatibility complex (MHC) antigens, respectively (S. Swain, Proc. Natl. Acad. Sci. **78**:7101 [1981]; E. Engleman *et al.*, J. Immunol. **127**:2124 [1981]; H. Spitz *et al.*, J. Immunol. **129**:1563 [1982]; W. Biddison *et al.*, J. Exp. Med. **156**:1065 [1982]; and D. Wilde *et al.*, J. Immunol. **131**:2178 [1983]). For the most part, CD4 T cells display the helper/inducer T cell phenotype (E. Reinherz, *supra*), although CD4 T cells characterized as cytotoxic/suppressor T cells have also been identified (Y. Thomas *et al.*, J. Exp. Med. **154**:459 [1981]; S. Meuer *et al.*, Proc. Natl. Acad. Sci. USA **79**:4395 [1982]; and A. Krensky *et al.*, Proc. Natl. Acad. Sci. USA **79**:2365 [1982]). The loss of CD4 helper/inducer T cell function probably underlies the profound defects in cellular and humoral immunity leading to the opportunistic infections and malignancies characteristic of AIDS (H. Lane *supra*).

The clinical spectrum of HIV infection is very complex, and the current therapy is directed toward the underlying retroviral infection as well as towards the specific opportunistic infections and malignancies that are associated with the syndrome.

Although the mechanism of HIV-associated ITP is unknown, it is believed to be different from the mechanism of ITP not associated with HIV infection in more characteristics.

In a study by Walsh *et al.* [N. Engl. J. Med. **311**, 635-639 (1984)] ITP in 33 homosexual men who were presumed to be HIV-positive, was compared with non-HIV-related chronic ITP in 15 women and eight men. Individuals with HIV-associated ITP were reported to have 3.8-fold higher levels of platelet associated IgG and 4.2-fold higher levels of platelet-associated complement than did the patients with non-HIV-related ITP. Platelet eluates from 12 of 15 patients with non-HIV-related ITP bound platelets at a mean dilution titer of 1:8, whereas those from only one of the 10 individuals with HIV-associated ITP bound platelets at a titer of 1:2. Also, IgG in the serum of three patients with HIV-associated ITP was not capable of binding platelets, whereas IgG from two patients with non-HIV-associated ITP could bind platelets. Circulating immune complexes (CICs) were detected by the polyethylene glycol precipitation method in 88 % of the individuals having HIV-associated ITP, and in 79 % the CICs were capable of binding to platelets. CICs were not found in any of five patients with non-HIV-associated ITP. These and similar findings have led to the hypothesis that HIV-related ITP results from the deposition of immune complexes and complement on platelets. The immune complex deposition occurs on platelet Fc receptors. Monocytes or macrophages would then bind to free Fc domains of exposed IgG molecules or to platelet-bound complement by the C3b receptors.

An alternative hypothesis is that HIV-associated ITP results from an autoimmune process associated with the presence of a serum antibody that binds to a target platelet antigen of 25,000 kilodaltons (kD). There is a suggestion that the 25 kD protein resembles part of a sequence from the HIV polypeptide. Molecular mimicry between HIV and platelet antigens may play a role in the induction of ITP in HIV-infected individuals. This hypothesis is essentially based on a study by Stricker *et al.*, N. Engl. J. Med. **313**, 1375-1380 (1985). The authors studied ITP in patients including 30 homosexual men, 18 non-homosexual individuals with ITP, 12 patients with nonimmune thrombocytopenia and 16 homosexual men with normal platelet counts diagnosed to have either AIDS-related complex (ARC) or AIDS. Using a sensitive immunoblot technique, an antibody was identified in the sera of 29 of 30 patients with HIV-associated ITP that bound to a 25 kilodalton antigen on platelets. This activity was not found in the sera of patients with non-HIV-associated ITP or nonimmune thrombocytopenia. The serum binding activity for the 25 kilodalton platelet antigen was found in the IgG fraction and was mediated by the F(ab)₂ fragment.

For further details, I refer to a recent review article by Ratner, L., [Am. J. Med. **86**, 194-198 (1989)], and the references cited therein.

The therapeutic approach to the treatment of patients with ITP is dictated by the severity and urgency of the clinical situation. The treatment is similar for HIV-associated and non-HIV-related ITP, and although a number of different therapeutic approaches have been used in the clinical practice, the therapy remains controversial.

In many adult patients with (HIV-associated) ITP thrombocytopenia is mild (the platelet count is more than about $20-30 \times 10^9/\text{liter}$) and no bleeding occurs. In such cases monitoring with no therapy is considered to be the best option.

Platelet counts in patients diagnosed with ITP have been successfully increased by glucocorticoid (e.g. prednisolone) therapy, however in most patients the response is incomplete, or relapse occurs when the glucocorticoid dose is reduced or its administration is discontinued. Based upon studies with patients having HIV-associated ITP, some investigators have suggested that glucocorticoids may predispose to AIDS. This approach is usually followed if the platelet count is below $20 \times 10^9/\text{liter}$ or if bleeding occurs.

Good results were reported with splenectomy. Splenectomy removes the major site of platelet destruction and a major source of autoantibody production in many patients. This procedure results in prolonged treatment-free remissions in a large number of patients. However, since surgical procedures are generally to be avoided in immune compromised patients, splenectomy is recommended in severe cases of HIV-associated ITP only, in patients who fail to respond to 2 to 3 weeks of glucocorticoid treatment, or do not achieve sustained response after discontinuation of glucocorticoid administration. Based upon current scientific knowledge, it is unclear whether splenectomy predisposes patients to AIDS.

Significant responses have been documented to high doses (about 500 mg/kg body weight and above) of immunoglobulin (Rarick *et al.*, Proceedings of the Fourth International Conference on AIDS, Stockholm, (1988) p. 7642; Sanderson *et al.*, *ibid.*, p. 7646; Panzer *et al.*, *Transfusion* 26, 69-72 (1986); Delfraissy *et al.*, *Ann. Intern. Med.* 103, 478-479 (1985); Ordi *et al.*, *Ann. Intern. Med.* 104, 282-283 (1986)), and to anti-Rh immunoglobulin treatment [Oksendhender *et al.*, *Blood* 71, 1499-1502 (1988); Bierling *et al.*, *Ann. Intern. Med.* 106, 773-774 (1987); Durand *et al.*, *Lancet* 2:40 (1986); Biniek *et al.*, *Lancet* 2:627 (1986)].

Certain cytotoxic agents, e.g. vincristine, and azidothymidine (AZT, zidovudine) have also shown some promising effects, however, the results are very preliminary.

AZT, a reverse transcriptase inhibitor nucleoside analogue, has been extensively tested in AIDS therapy and was found to result in a decreased incidence of opportunistic infections and increased survival in AIDS patients [Fischl *et al.*, *N. Engl. J. Med.* 317, 185-191 (1987)]. However, it is clear that AZT therapy is associated with significant dose-limiting toxicities, including anemia and other hematologic toxicity, particularly in individuals with advanced HIV infection [Richman *et al.*, *N. Engl. J. Med.* 317, 192-197 (1987)]. Similarly, the toxicity of cytotoxic agents, such as vincristine is of great concern.

CD4-Immunoglobulin hybrid molecules are known in the art, and are, for example, disclosed in co-pending U. S. Application Serial No. 07/250,785 (filed 28 September 1988), which is a continuation-in-part of Serial No. 07/104,329 (filed 2 October 1987), and in the counterpart PCT Application Publication No. WO 89/02922 (published 6 April 1989). Fusion proteins of immunoglobulins of the IgM, IgG1 or IgG3 class, wherein the variable region of the light or heavy chain has been replaced with CD4 or CD4 fragments are also described in EP 325,262. Such fusion proteins have so far been recommended for the treatment of HIV infection or for use in assays for detection of the HIV virus. According to WO 89/02922 the typical dose for the treatment of HIV infection corresponds to about 100 μ g/kg/day soluble CD4. According to EP 325,262 the dose of the CD4-immunoglobulin molecule may vary from 0.01 mg/kg to 50 mg/kg, 0.1 mg/kg to 1.0 mg/kg being preferred.

The results of a phase 1 clinical study initiated to evaluate the safety and pharmacokinetics of a recombinant soluble CD4 (rCD4) molecule in the treatment of AIDS were reported by Kahn *et al.* in *Annals of Internal Medicine* 112(4), 254-261 (1990). The patients were enrolled at dose levels of 1, 10, 30, 100, and 300 μ g/kg body weight of rCD4 administered intravenously. One of the patients had a platelet count of 68×10^9 /liter at entry, and during the rCD4 treatment had a progressive decrease to 41×10^9 /liter at the time that disseminated histoplasmosis with bone marrow involvement was diagnosed.

It is an object of the present invention to provide an efficient treatment for HIV-associated ITP which is devoid of the disadvantages of the hitherto known approaches.

Summary of the Invention

The object of the present invention is accomplished by providing a method for treating HIV-associated immune thrombocytopenic purpura (ITP) which comprises administering to a patient in need of such treatment a therapeutically effective amount of a molecule comprising an amino acid sequence capable of binding to HIV.

5 In a further aspect, the present invention concerns a method for treating HIV-associated ITP which comprises:

- a) administering to a patient having the condition of HIV-associated ITP a therapeutically effective amount of a molecule comprising an amino acid sequence capable of binding to HIV;
- 10 b) monitoring the change in a parameter or symptom characteristic of the condition of HIV-associated ITP in response to the administration; and
- c) continuing the treatment until a positive response is attained.

The amino acid sequence capable of binding to HIV may, for example, be a CD4 or an immunoglobulin variable domain sequence, optionally fused to a carrier amino acid sequence which is preferably an immunoglobulin constant domain sequence.

15 According to a particular embodiment, the treatment is performed with a hybrid molecule comprising a CD4 sequence which is capable of binding to HIV, said sequence being fused to an immunoglobulin constant domain sequence. Typically, the CD4 sequence is a portion of the extracellular domain of the CD4 receptor. The immunoglobulin sequence may be obtained from IgG-1, -2, -3, or -4 subtypes, IgA, IgE, IgD or IgM, and preferably is a portion of human immunoglobulin of the IgG-1 subclass.

20 In another embodiment, anti-HIV monoclonal antibodies are used in the above method.

Brief Description of the Drawings

25 Figure 1 illustrates the structure of the human soluble recombinant CD4-immunoglobulin (rCD4-IgG-1) hybrid molecule used in the example.

Figures 2 and 3 are graphical illustrations of the results recorded in two series of clinical trials. The platelet counts are shown as a function of the time of treatment.

30 Detailed Description

HIV is a retrovirus, containing three regions encoding structural proteins. The gag region encodes the core proteins of the virion, the pol region encodes the virion reverse transcriptase, and the env region encodes the major glycoprotein found in the membrane envelope of the virus and in the cytoplasmic membrane of the cells infected with the HIV virus. The structural element which is believed to play a fundamental role in the pathogenesis of the virus is the env region encoding a precursor env (envelope) polypeptide gp160. Cleavage of this precursor yields gp120 (a heavily glycosylated exterior membrane protein of about 481 amino acids) and gp41 (a transmembrane protein of about 345 amino acids).

For further details about the structure and complete nucleotide sequence of HIV see, e.g. Ratner *et al.*, Nature **313**, 277-285 (1985).

Molecules comprising an amino acid sequence capable of binding to any domain within the env region including the gp160, gp120 and gp41 regions, are all contemplated as being
5 useful for the purpose of the present invention.

A molecule known to bind to gp120 is the cell surface glycoprotein, CD4.

The known sequence of the CD4 precursor predicts a hydrophobic signal peptide, an extracellular region of approximately 370 amino acids, a highly hydrophobic stretch with significant identity to the membrane-spanning domain of the class II MHC beta chain, and a
10 highly charged intracellular sequence of 40 residues [P. Maddon, Cell **42**, 93 (1985)]. Because of its amino acid sequence homology with immunoglobulin polypeptide chains, CD4 is considered to be a member of the immunoglobulin gene superfamily.

Detailed molecular studies of the CD4 molecule indicate that it is a transmembrane protein. The extracellular domain of CD4 consists of four contiguous regions each having
15 amino acid and structural similarity to the variable and joining (V-J) domains of immunoglobulin light chain as well as related regions in other members of the immunoglobulin gene superfamily. These structurally similar regions of CD4 are termed the V1, V2, V3 and V4 domains. The boundaries for the CD4 V-like regions (V1-V4) are, respectively, about 100-109, about 175-184, about 289-298, and about 360-369, based on the precursor CD4 amino
20 acid sequence in which the initiating met is -25, as disclosed by Arthos *et al.*, Cell **57**, 469 (1989).

Truncation studies of the CD4 molecule have been conducted to identify the region to which the viral gp 120 actually binds. Results of these studies suggest that the V1 region, consisting of approximately the first 100 amino acids of the extracellular domain, is alone
25 sufficient for binding of gp 120 [Richardson *et al.*, Proc. Nat'l. Acad. Sci. USA **85**, 9273 (1988); Landau *et al.*, Nature **334**, 159 (1988)]. Several approaches have been used to define the amino acids within the V1 region to which gp 120 binds. These approaches include the techniques of random saturation mutagenesis coupled with selection of escape mutants [Peterson and Seed, Cell **54**, 65 (1988) and EP 342,444 A2 published 15 November
30 1989]; insertional mutagenesis [Mizukami *et al.*, Proc. Nat'l. Acad. Sci. USA **85**, 9273 (1988)]; homolog-scanning mutagenesis (replacement of sequences from human CD4 which binds gp 120, with unconserved sequences from mouse CD4, which does not bind gp 120) [Landau *et al.*, Nature **334**, 159 (1988); Clayton *et al.*, Nature **335**, 363 (1988); WO 89/0322, published 20 April 1989; and Brodsky *et al.*, J. Immunol. **144**, 3078 (1990)]; and
35 alanine scanning mutagenesis [Ashkenazi *et al.*, Proc. Nat'l. Acad. Sci. USA **87**, 7150 (1990)].

The term "CD4" in the context of the present invention is used to refer to a polypeptide comprising the sequence of the native human CD4 molecule as disclosed by

- Arthos *et al.*, Supra, or any derivative or fragment thereof which is effective in the treatment of ITP. This definition includes CD4 polypeptides from natural source, synthetically produced or obtained by genetic manipulation including methods of recombinant DNA technology, and specifically encompasses the sequence initially published by Maddon *et al.*, Supra, which
- 5 indicates an asparagine residue (codon AAC) at the 26 position (position 1 of mature CD4), although it has later been found that the sequence which occurs in nature contains a lysine residue at this position (encoded by AAG). "CD4 derivatives" as defined for the purpose of the present invention, specifically include CD4 molecules having the qualitative gp 120 binding characteristics of native human CD4 and comprising a gp 120 binding domain of CD4.
- 10 This definition includes alleles, fragments (truncated versions) and insertional and deletional sequence variants of native CD4 (see co-pending U.S. application Serial No. 07/512,691 filed 23 April 1990). In a particular group of such CD4 derivatives at least the transmembrane domain is inactivated so that it is incapable of cell membrane insertion. This is typically accomplished by deletion of the transmembrane domain, optionally including deletion of the
- 15 cytoplasmic domain and all extracellular sequence located downstream from the first two variable region-like domains of CD4. The CD4 polypeptides are lysine N-terminal (the native sequence) or are N-terminated by asparagine or other suitable residues in place of lysine. CD4 derivatives are, for example disclosed in the U. S. Application Serial No. 07/104,399 filed 26 October 1987 (now abandoned), its Continuation-In-Part Serial No. 07/250,785 filed
- 20 28 September 1988 (PCT WO 89/02922, published 6 April 1989), its Continuation-In-Part Serial No. 07/275,296 filed 23 November 1988 (PCT WO 90/05534, published 31 May 1990) and its Continuation-In-Part Serial No. 07/512,691 filed 23 April 1990. Further CD4 derivatives which qualify for the purpose of the present invention are easily identified by using the test method disclosed in the Example of the present application.
- 25 CD4 is a glycoprotein, which is defined as a polypeptide comprising a carbohydrate substituent. In glycoproteins, the carbohydrate typically is a branched polysaccharide containing fucose, N-acetylglucosamine, galactose, mannose, N-acetylneuraminic acid (sialic acid) and other sugar residues. The carbohydrate is substituted at N-linked glycosylation sites (asp X thr/ser, where X is any residue) or, in other polypeptides, at O-linked sites (typically
- 30 serine) or at both O and N-linked sites. It will be understood that CD4 from other recombinant hosts may contain different sugars or may vary in the relative proportions of the sugars shown above. It is within the scope hereof to move, add or delete glycosylation sites by site-directed mutagenesis of CD4 polypeptide either used alone or as a component of the CD4-immunoglobulin hybrids, in order to modify their properties.
- 35 According to a preferred embodiment of the invention, CD4 is conjugated to an immunoglobulin (Ig) constant domain to yield CD4-immunoglobulin hybrid molecules. Such molecules have a longer half-life and lower clearance than CD4 molecules alone, producing

much higher steady-state CD4 concentrations for equivalent doses [Capon *et al.*, Nature **337**, 525-531 (1989)].

Immunoglobulins and certain variants thereof are known and many have been prepared in recombinant cell culture. For example, see U.S. Patent 4,745,055; EP 256,654; Faulkner
5 *et al.*, Nature **298**:286 (1982); EP 120,694; EP 125,023; Morrison, J. Immun. **123**:793 (1979); Köhler *et al.*, Proc. Nat'l. Acad. Sci. USA **77**:2197 (1980); Raso *et al.*, Cancer Res. **41**:2073 (1981); Morrison *et al.*, Ann. Rev. Immunol. **2**:239 (1984); Morrison, Science **229**:1202 (1985); Morrison *et al.*, Proc. Nat'l. Acad. Sci. USA **81**:6851 (1984); EP 255,694; EP 266,663; and WO 88/03559. Reassorted immunoglobulin chains also are known. See
10 for example U.S. patent 4,444,878; WO 88/03565; and EP 68,763 and references cited therein.

The term "CD4-immunoglobulin hybrid" and grammatical variants thereof as defined for the purpose of the present invention refer to molecules comprising a CD4 moiety as hereinabove defined conjugated to an immunoglobulin constant domain. Ordinarily, the CD4
15 moiety is linked at its C-terminus to the N-terminus of the constant region of an immunoglobulin in place of the variable region(s) thereof, retaining at least the CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain and preferably, a functionally active hinge. This ordinarily is accomplished by constructing the appropriate DNA sequence and expressing it in recombinant cell culture.

20 In a specific group, a CD4 sequence containing the CD4 HIV-binding V-like regions is fused to the immunoglobulin sequence. It is preferable that the V1, V1V2 or V1V2V3V4 regions be fused at their C-termini to the N-terminus of an immunoglobulin constant region. The precise site at which the fusion is made is not critical; the boundary domains noted herein are for guidance only and other sites neighboring or within the V regions may be
25 selected in order to optimize the secretion or binding characteristics. The optimal site can be determined by routine experimentation. In general, it has been found that the fusions are expressed intracellularly, but a great deal of variation is encountered in the degree of secretion of the fusions from recombinant hosts. The following table demonstrates a number of various immunoglobulin fusions that have been made as examples of structures that are
30 believed to be suitable for the purpose of the present invention. In all examples, the CD4 signal was used to direct secretion from 293 cells. Lower case m represents murine origin, while the lower case h designates human origin. V and C are abbreviations for immunoglobulin variable and constant domains respectively. The numerical subscripts indicate the number of parenthetical units found in the designated multimer. It will be
35 understood that the chains of the multimers are believed to be disulfide bonded in the same fashion as native immunoglobulins. The CD4 immunoglobulin hybrids typically contain either the first N-terminal 366 residues of CD4 (CD4₄) or the first 180 N-terminal residues of CD4

(CD4₂) linked at their C-terminus to the κ (light) chain or IgG-1 heavy chain constant region (γ 1).

Table I

	<u>Transfected Gene</u>	<u>Secreted Product</u>
5	hCD4-mC _{κ}	hCD4-mC _{κ} and/or (hCD4-mC _{κ}) ₂
	hCD4-mC _{κ} + hCD4-mC _{γ1}	(hCD4-mC _{κ}) ₂ (hCD4-mC _{γ1)₂ + hCD4-mC_{κ} and/or (hCD4-mC_{κ})₂}
10	hCD4-hC _{κ}	hCD4-hC _{κ} and/or (hCD4-hC _{κ}) ₂
	hCD4-hC _{γ1}	(hCD4-hC _{γ1)₂}
15	hCD4-hC _{κ} + hCD4-hC _{γ1}	(hCD4-hC _{κ}) ₂ (hCD4-hC _{γ1)₂ + hCD4-hC_{κ} and/or (hCD4-hC_{κ})₂}
	mV _{κ} C _{κ} + hCD4-hC _{γ1}	(mV _{κ} C _{κ}) ₂ (hCD4-hC _{γ1)₂ + mV_{κ}C_{κ} and/or (mV_{κ}C_{κ})₂}

20

CD4-IgG hybrids (chimeras) are readily secreted wherein the CD4 epitope is present in heavy chain dimers, light chain monomers or dimers, and heavy and light chain heterotetramers wherein the CD4 epitope is present fused to one or more light or heavy chains, including heterotetramers wherein up to and including all four variable region analogues are derived from CD4. Where a light and/or heavy chain non-CD4 variable domain is present, a heterofunctional antibody thus is provided. Typically, a heavy-light chain heterodimer capable of binding a predetermined antigen is disulfide bonded in the ordinary fashion through the heavy chain constant domain to a CD4-heavy chain chimera. The predetermined antigen can be a platelet antigen or a viral (e.g. HIV) antigen.

25

30

The preparation of the particular CD4-IgG-1 hybrid used in our clinical trials is described in the Example. The term "(human) rCD4-IgG-1" as used throughout the specification and claims refers to this specific hybrid.

35

In general, the hybrid molecules used in accordance with the present invention are constructed in a fashion similar to chimeric antibodies in which a variable domain from an antibody of one species is substituted for the variable domain of another species. See, for example, EP 0 125 023; Munro, Nature 312: (13 December 1984); Neuberger *et al.*, Nature 312: (13 December 1984); Sharon *et al.*, Nature 309: (24 May 1984); Morrison *et al.*, Proc. Nat'l. Acad. Sci. USA 81:6851-6855 (1984); Morrison *et al.*, Science 229:1202-1207 (1985); and Boulianne *et al.*, Nature 312:643-646 (13 December 1984). The DNA encoding the desired CD4 immunoglobulin-like domain(s) is cleaved by a restriction enzyme at or

40

proximal to the 3' end of the DNA encoding the immunoglobulin-like domain(s) and at a point at or near the DNA encoding the N-terminal end of the mature CD4 polypeptide (where use of a different leader is contemplated) or at or proximal to the N-terminal coding region for CD4 (where the native signal is employed). This DNA fragment then is readily inserted
5 proximal to DNA encoding an immunoglobulin light or heavy chain constant region and, if necessary, the resulting construct tailored by deletional mutagenesis. Preferably, the Ig is a human immunoglobulin when the variant is intended for *in vivo* therapy for humans. DNA encoding immunoglobulin light or heavy chain constant regions is known or readily available from cDNA libraries or is synthesized. See for example, Adams *et al.*, Biochemistry **19**:2711-
10 2719 (1980); Gough *et al.*, Biochemistry **19**:2702-2710 (1980); Dolby *et al.*, P.N.A.S. USA, **77**:6027-6031 (1980); Rice *et al.*, P.N.A.S. USA **79**:7862-7865 (1982); Falkner *et al.*, Nature **298**:286-288 (1982); and Morrison *et al.*, Ann. Rev. Immunol. **2**:239-256 (1984).

According to an another embodiment of the present invention, HIV-associated ITP is treated with a molecule comprising an immunoglobulin variable region. Antibodies (specific
15 immunoglobulin polypeptides with specificity for an HIV antigen), and specifically monoclonal antibodies to gp120, are particularly suitable for this purpose. As used herein, the term "antibodies" refers to tetramers or aggregates thereof which have specific immunoreactive activity, comprising light and heavy chains usually aggregated in an "Y" configuration, with or without covalent linkage between them; whereas the term "immunoglobulins" refers to
20 such assemblies whether or not specific immunoreactive activity is a property.

Methods for the production of monoclonal antibodies are well known in the art (Kohler *et al.* [Eur. J. Immunol. **6**, 511 (1976)]; Current Protocols in Molecular Biology, Ausubel, F.M. *et al.* Eds., Greene Publishing Associates and Wiley-Interscience, 1988, Vol. 2). In this process, splenocytes or lymphocytes from a mammal injected with an antigen are fused with
25 a tumor cell line, to yield hybridomas which are both immortal and capable of producing the genetically coded antibody of the B cell. The hybrids are segregated into single genetic strains by selection, dilution, and regrowth, and each strain thus represents a single genetic line. They therefore produce immunoreactive antibodies against a desired antigen which are assured to be homogenous, and which antibodies, referencing their pure genetic parentage, and called "monoclonal". Alternatively, antibodies can be produced by using techniques of
30 recombinant DNA technology. Recombinant techniques are suitable for producing antibodies which are analogous to those normally found in vertebrate systems, and also for constructing chimeric or other modified forms, specifically designed for a particular application [see, e.g. U.S. Patent No. 4,816,567 and the references cited hereinabove for the construction of
35 chimeric antibodies]. Recombinant techniques can, for example, produce antibodies with a CD4 domain, structurally analogous to the CD4-immunoglobulin hybrids discussed hereinabove. The use of such recombinant antibodies for the treatment of HIV-associated ITP is within the scope of the present invention.

The compounds of the present invention are administered in the form of conventional pharmaceutical formulations, in association with non-toxic, pharmaceutically acceptable ingredients. Such formulations can be prepared by known methods described, for example, in Remington's Pharmaceutical Sciences, 16th Ed. 1980, Mac Publishing Company, and are typically administered parenterally by injection. Whereas intravenous, intraperitoneal, intramuscular and subcutaneous routes of administration are possible, the intravenous route is preferred.

The molecules comprising an amino acid sequence capable of binding to HIV are administered in a therapeutically effective amount. The term "therapeutically effective amount" is used to define an amount resulting in the improvement of any parameters or clinical symptoms characteristic of the condition of ITP, including bleeding symptoms and platelet count depression. A positive change in such parameters or symptoms, e.g. an increase in platelet count or the moderation or elimination of bleeding symptoms are considered a positive response. If the monitoring is based on the evaluation of the platelet counts, "complete response" is defined as the increase of the platelet count to at least 150,000 cells/mm³, whereas "partial response" refers to a platelet count less than 150,000 cells/mm³ and a greater than or equal to 30,000 cells/mm³ increase from baseline (i.e. the platelet count prior to treatment). However, other (or additional) methods, such as the detection of platelet-associated immunoglobulin by fluorescence-activated flow cytometric assay, bone marrow examination, platelet survival can also be used for monitoring the response of the ITP patients to therapy.

The actual dose will be different for the various specific molecules, and will vary with the patient's overall condition, the seriousness of the symptoms, counterindications, etc. The determination of the effective dose is well within the skill of a practicing physician. For the CD4-Ig conjugates the daily dosage is typically higher than about 1 mg/kg of body weight, and preferably is in the range of about 1 and about 20 mg/kg of body weight, more preferably between about 1 and about 10 mg/kg of body weight. Compounds comprising a CD4 sequence not linked to an immunoglobulin sequence are administered in higher doses, and more frequently due to the short half-life and rapid clearance of CD4.

Further details of the invention are illustrated in the following non-limiting Example.

Example

Study of the effect of recombinant human CD4-Immunoglobulin G-1 (rCD4-IgG-1) on HIV-associated ITP

Materials and Methods

The rCD4-IgG-1 hybrid used in this study was a homodimer of a hybrid polypeptide consisting of residues 1-180 of the mature human CD4 protein fused to human IgG-1

sequences beginning at aspartic acid 216 (taking amino acid 114 as the first residue of the heavy chain constant region [Kabat *et al.*, Supra]) which is the first residue of the IgG-1 hinge after the cysteine residue involved in heavy-light chain bonding, and ending with residue 441. This antibody-like molecule, the structure of which is illustrated in Figure 1, contains two N-terminal CD4 domains, a hinge region and the C_H2 and C_H3 Fc domains. The C_H2 domain contains the only potential N-linked glycosylation site on the molecule. The CD4-IgG-1 hybrid shown in Figure 1, which lacks the CH1 domain, was derived from a CH1-containing CD4-IgG-1 hybrid (immunoadhesin) the preparation of which was described by Capon *et al.*, Nature 337, 525-531 (1989) and is disclosed in the PCT Patent Application Publication No. WO 89/02922 (published 6 April 1989) claiming the priorities of USSN 07/104,329 (filed 20 October 1987) and USSN 07/250,785 (filed 28 September 1988). The present (CH1-lacking) hybrid was obtained by oligonucleotide-directed deletional mutagenesis [Zoller, M. & Smith, M., Nucleic Acids Res. 10, 6487-6500 (1982)], and expressed in Chinese hamster ovary (CHO) cells after their insertion into a mammalian expression vector used for soluble rCD4 expression [Smith *et al.*, Science 238, 1704-1707 (1987)]. The product was purified to a purity exceeding 99% using protein A-Sepharose chromatography, as described by Capon *et al.*, Supra.

To prepare a pharmaceutical formulation, the rCD4-IgG-1 hybrid was diluted with sufficient formulation buffer to produce a 5.0 mg/ml concentration of rCD4-IgG. The formulation buffer consisted of 0.16 mg/ml citric acid monohydrate, 2.7 mg/ml trisodium citrate, 41 mg/ml mannitol, 1 mg/ml glycine and 1 mg/ml polysorbate 20. The solution obtained was lyophilized, and the lyophilized powder was filled into vials with a nominal content of 5 mg and 25 mg rCD4-IgG-1, which were stored under refrigeration. Immediately before administration, the 5 mg and 25 mg vials of rCD4-IgG-1 were reconstituted with 1.2 ml and 5.5 ml of Sterile Water-for-Injection, USP, respectively, to result in a concentration of 5 mg/ml.

Study Design

The safety and efficacy of rCD4-IgG-1 in patients with HIV-associated thrombocytopenic purpura (ITP) were studied. The study has been performed on male and female subjects, 18 years or older. Patients were selected to meet the following criteria:

1. Patients were required to have a platelet count of 100,000/mm³ or less on two successive occasions done one week apart. Patients with platelet count \leq 10,000/mm³ were excluded from the study.
2. Previously documented HIV-1 seropositivity.
 - a. Patients with AIDS (using the diagnostic criteria of the Centers for Disease Control) must have had no more than 2 prior episodes of pneumocystis carinii pneumonia, and no more than one prior episode of another serious opportunistic infection.

b. Patients with HIV infection without a diagnosis of AIDS and not asymptomatic: They must have had a history of one of the following symptoms: thrush, oral hairy leukoplakia, fevers, night sweats, weight loss, diarrhea, fatigue, dermatitis.

c. Asymptomatic patients.

5 The enrolled patients had otherwise normal hepatic, hematologic, coagulation and renal function. Patients previously treated with zidovudine were allowed to remain on this treatment. Subjects with active serious opportunistic infections, significant neurologic, cardiac or liver disease were excluded from this study.

10 Post study evaluation was performed within 3-7 days of the completion of the study or withdrawal from treatment. The response criteria were as follows:

1. Complete response was a platelet count of at least 150,000 cells/mm³.

2. Partial response was a platelet count less than 150,000 cells/mm³ and a greater than or equal to 30,000 cells/mm³ increase from baseline.

3. No response was less than a 30,000 cells/mm³ increase from baseline.

15 After completion of the initial 8-week phase of the protocol, patients who responded to the treatment and showed no evidence of toxicity were allowed to stay on maintenance therapy. Maintenance therapy is initiated within 2 weeks of completion of the initial 8 weeks of study and involves the administration of a 75 mg per infusion unit dose of rCD4-IgG two times a week.

20

Clinical and Laboratory Monitoring

At entry and throughout the study, subjects were evaluated clinically with interval histories, vital signs, and physical examinations. Safety monitoring by laboratory evaluation at regularly scheduled intervals included complete blood count with differential, platelet count, and reticulocyte count, chemistry profile, including albumin, alkaline phosphatase, total
25 bilirubin, blood urea nitrogen, calcium, cholesterol, triglycerides, glucose, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, sodium, potassium, and uric acid; routine urine analysis; coagulation profile; chest radiograph; and electrocardiogram, and (for female patients) pregnancy test.

30

Results

Figure 2 illustrates the results obtained on six subjects treated with rCD4-IgG-1 at a fixed dose level of 1 mg/kg by intravenous bolus injection on days 1 through 7, followed by intravenous boluses two times per week for the indicated time. One patient had a partial
35 response with platelet counts above 100,000. rCD4-IgG-1 was well tolerated by all subjects with insignificant clinical toxicity.

Figure 3 illustrates the results of another series of tests, where four subjects were treated at a fixed dose level of 6 mg/kg, under otherwise similar conditions. As shown in the

figure, three patients responded to the treatment with a striking increase in their platelet counts.

5 Although the foregoing refers to particular preferred embodiments, it will be understood that the present invention is not so limited. It will occur to those ordinarily skilled in the art that various modifications may be made to the disclosed embodiments without diverting from the overall concept of the invention. All such modifications are intended to be within the scope of the present invention.

CLAIMS:

1. A method for treating human immunodeficiency virus (HIV)-associated immune thrombocytopenic purpura (ITP) which comprises administering to a patient in need of such treatment a therapeutically effective amount of a molecule comprising an amino acid sequence capable of binding to HIV.
5
2. The method of Claim 1 wherein the platelet count of said patient prior to treatment is lower than about 150×10^9 per liter.
3. The method of Claim 2 wherein said amino acid sequence capable of binding to HIV is the HIV-binding domain of CD4.
10
4. The method of Claim 3 wherein said molecule is a CD4-immunoglobulin hybrid molecule, comprising a fusion of an HIV-binding domain of CD4 to an immunoglobulin constant domain sequence.
5. The method of Claim 4 wherein the immunoglobulin sequence is obtained from an IgG immunoglobulin.
- 15 6. The method of Claim 2 wherein said amino acid sequence capable of binding to HIV is a variable domain sequence of an anti-HIV monoclonal antibody.
7. A method for treating HIV-associated ITP which comprises:
 - a) administering to a patient having the condition of HIV-associated ITP a therapeutically effective amount of a molecule comprising an amino acid sequence capable
20 of binding to HIV;
 - b) monitoring the change in a parameter or symptom characteristic of the condition of HIV-associated ITP in response to the administration; and
 - c) continuing the treatment until a positive response is attained.
8. The method of Claim 7 wherein said parameter is the platelet count.
- 25 9. The method of Claim 8 wherein the platelet count of the patient prior to treatment is between about 100×10^9 per liter and about 10×10^9 per liter.
10. The method of Claim 9 wherein the criteria for positive response is a platelet count of at least 150,000 cells/mm³ or an at least 30,000 cells/mm³ increase from the platelet count prior to treatment.
- 30 11. The method of Claim 10 wherein said amino acid sequence capable of binding to HIV is the HIV-binding domain of a CD4 sequence.
12. The method of Claim 11 wherein said amino acid sequence comprises a CD4 V1 domain.
13. The method of Claim 12 wherein said molecule is a CD4-immunoglobulin hybrid
35 molecule, comprising a fusion of an HIV-binding domain of CD4 to an immunoglobulin constant domain sequence.
14. The method of Claim 13 wherein said hybrid molecule comprises a constant domain sequence of an immunoglobulin heavy chain.

15. The method of Claim 14 wherein the constant domain is linked at its N-terminus to the C-terminus of a transmembrane-deleted CD4.

16. The method of Claim 15 wherein said hybrid molecule comprises the fusion of a CD4 V-like domain to an immunoglobulin heavy chain constant domain containing the hinge, CH2 and CH3 domains.

17. The method of Claim 15 wherein the immunoglobulin sequence is obtained from IgG-1, -2, -3, or -4 subtypes, IgA, IgE, IgD or IgM.

18. The method of Claim 17 wherein the immunoglobulin sequence is obtained from IgG-1.

19. The method of Claim 18 wherein said hybrid is human rCD4-IgG-1.

20. The method of Claim 19 wherein said hybrid is administered in a dose of about 1 mg/kg of body weight to about 20 mg/kg of body weight.

1 / 3

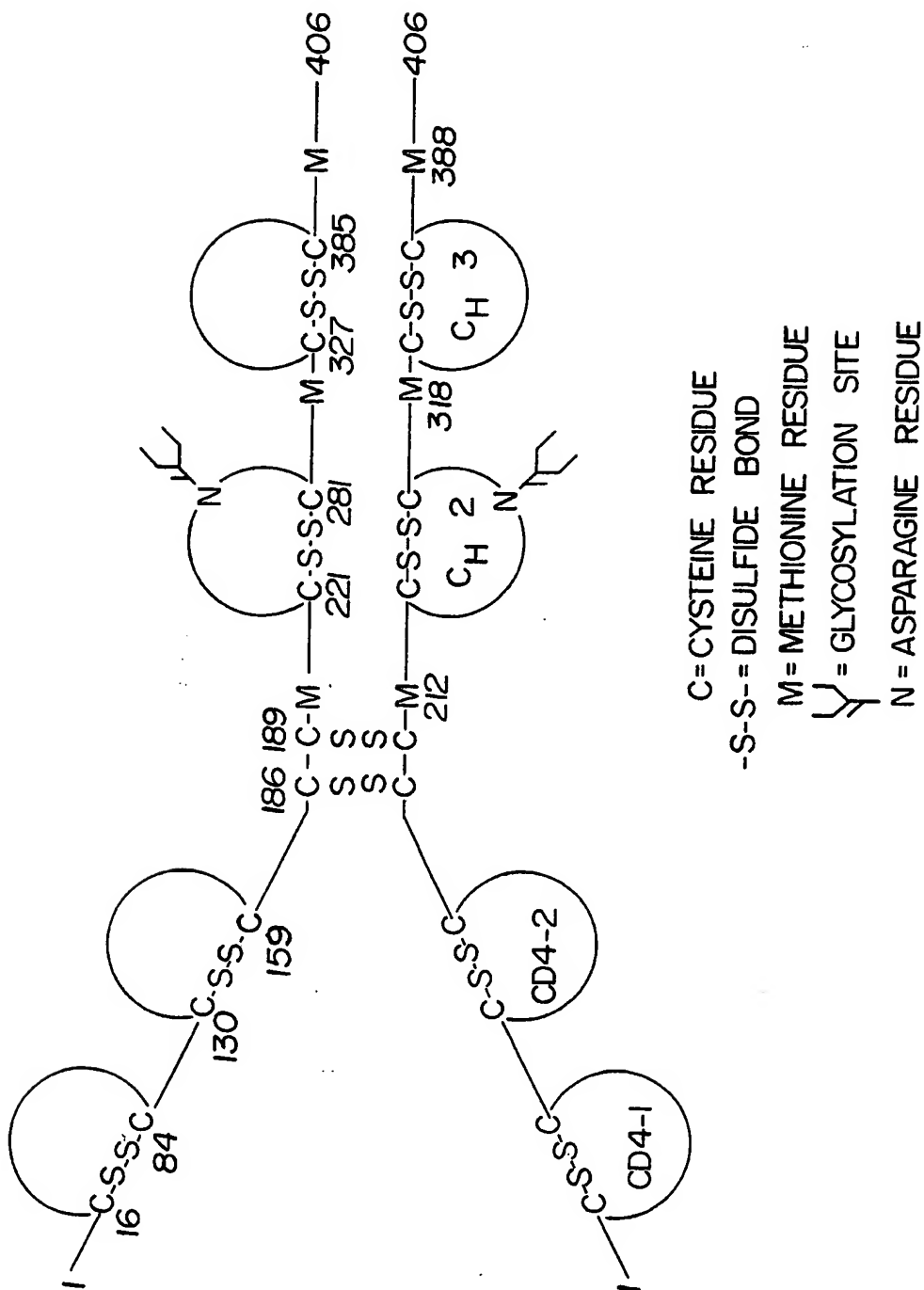


FIG. 1

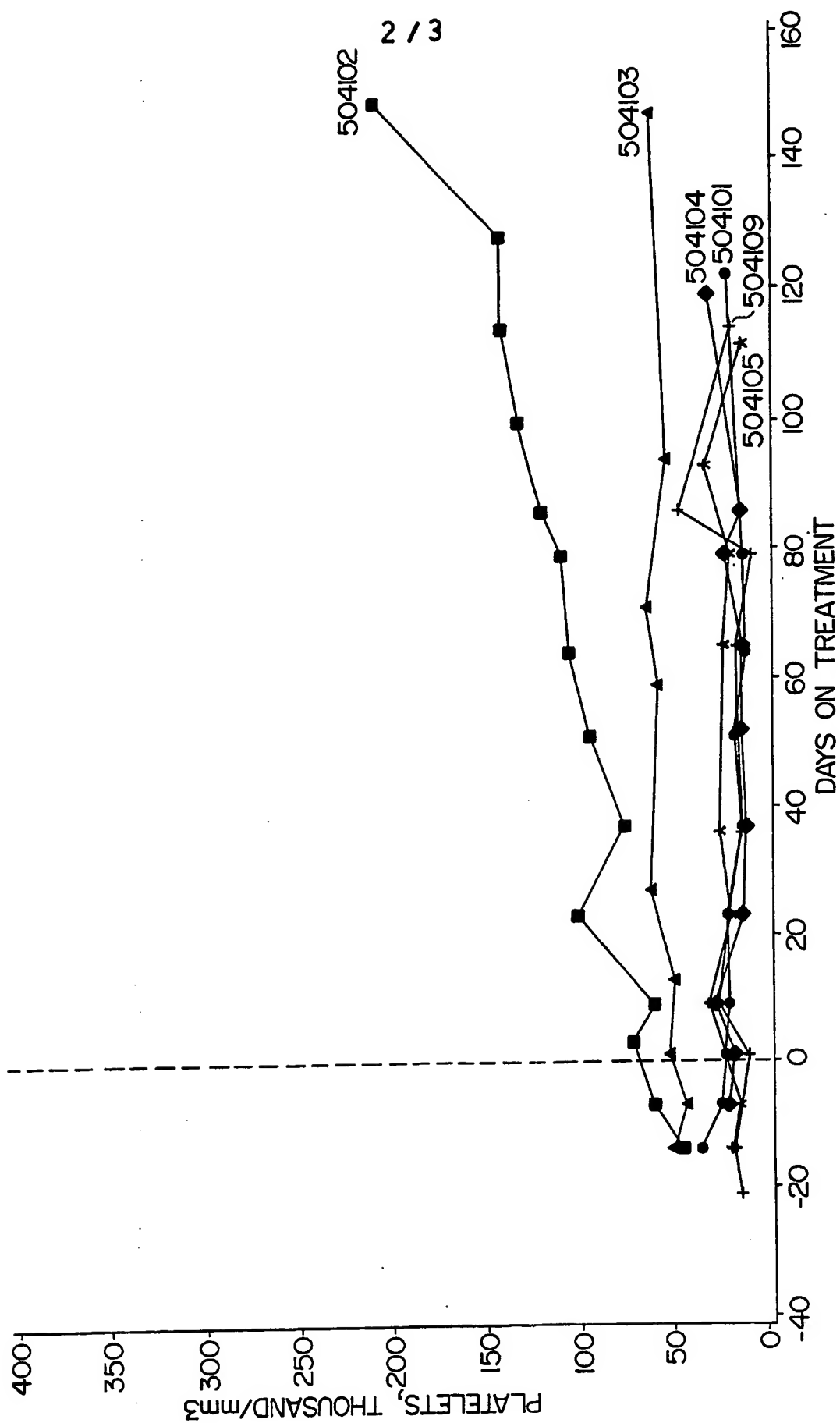


FIG. 2

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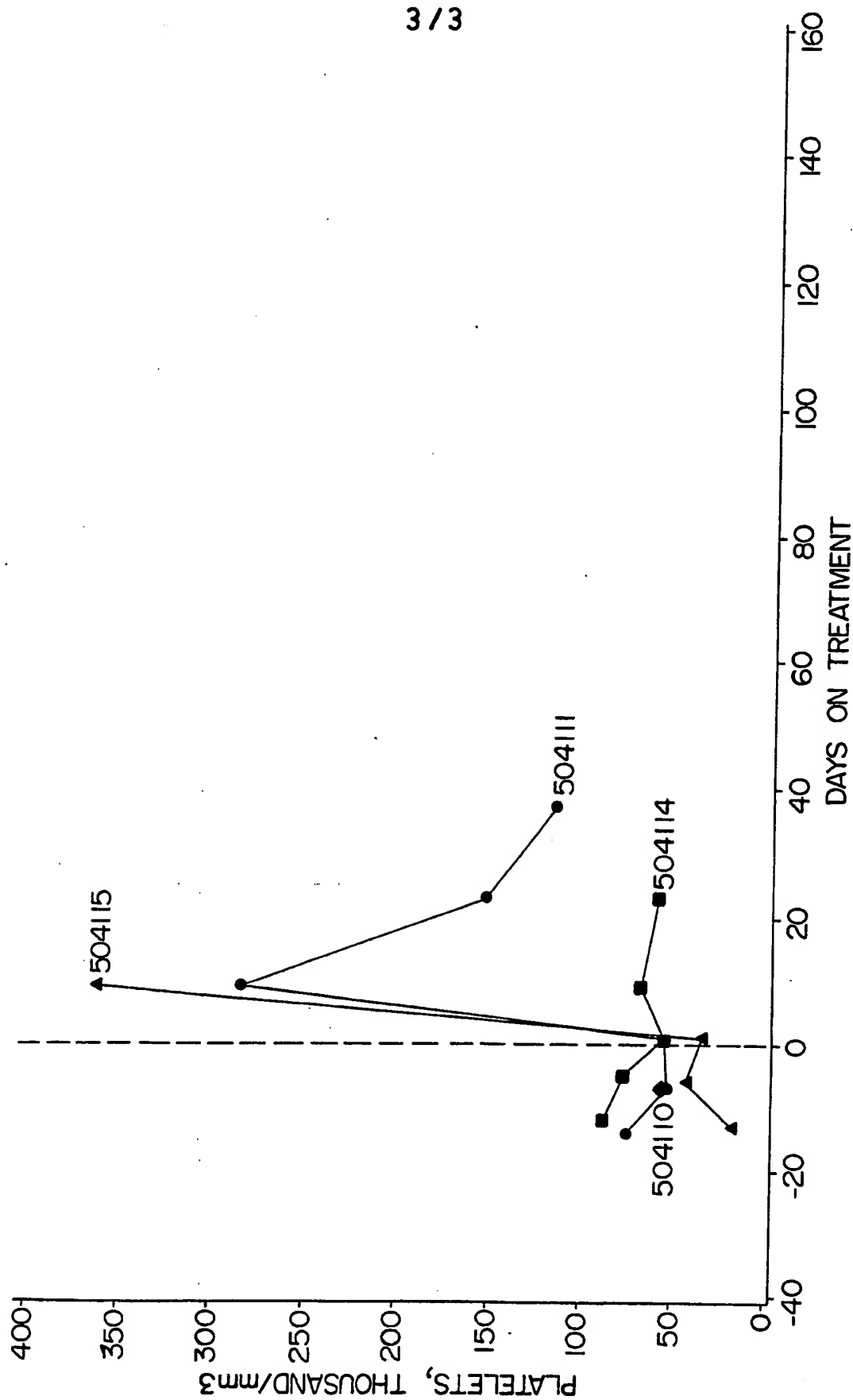
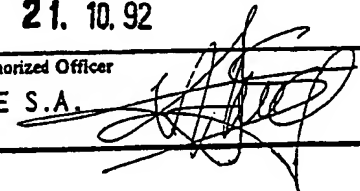


FIG. 3

INTERNATIONAL SEARCH REPORT

PCT/US 92/03138

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12N15/12; A61K39/42;	C07K13/00; A61K37/02	C12N15/62; A61K39/21
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C07K ; C12N ; A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	EP,A,0 325 262 (THE GENERAL HOSPITAL CORPORATION) 26 July 1989 cited in the application See pages 2-7; page 8, lines 1-30; page 10, lines 24-26; claims ---	1-19
X	EP,A,0 394 827 (F. HOFFMANN-LA ROCHE AG) 31 October 1990 See page 2; page 3, lines 1-39; page 6, lines 31-41; claims ---	1-19
X	EP,A,0 314 317 (GENENTECH, INC.) 3 May 1989 See pages 1-12; claims ---	1-19
X	WO,A,9 000 617 (BIO-RESEARCH LABORATORIES) 25 January 1990 See the abstract; claim 18 ---	1,6
-/--		
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
07 OCTOBER 1992	21. 10. 92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	NAUCHE S.A. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claim No.
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	
X	EP,A,0 385 909 (MICROGENESYS, INC.) 5 September 1990 see claims 1-3,5,6 ---	1-5,7-19
A	IMMUNOLOGICAL INVESTIGATIONS vol. 20, no. 2, April 1991, pages 223 - 229 Nydegger UE;Castelli D 'Review on therapeutic options in HIV associated thrombocytopenia with emphasis on i.v. immunoglobulin treatment.' see the whole document -----	1-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 92/03138

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 20
because they relate to subject matter not required to be searched by this Authority, namely:
see PCT-Rule 39.1(iv).
Remark: Although claims 1-19 are directed to a method of treatment of the human body (Rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. US 9203138
SA 60690**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 07/10/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0325262	26-07-89	AU-B- 611551	13-06-91
		AU-A- 3281889	11-08-89
		JP-T- 3502283	30-05-91
		WO-A- 8906690	27-07-89

EP-A-0394827	31-10-90	JP-A- 3035781	15-02-91

EP-A-0314317	03-05-89	AU-A- 2557188	18-04-89
		EP-A- 0383799	29-08-90
		JP-T- 4502850	28-05-92

WO-A-9000617	25-01-90	EP-A- 0423198	24-04-91
		JP-T- 3505668	12-12-91

EP-A-0385909	05-09-90	AU-A- 5061090	01-11-90
		CA-A- 2011299	03-09-90
		JP-A- 3163027	15-07-91
